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## ORIGINAL ARTICLE

# Comparative Study of *In Vitro* Antioxidant Potential of Biosynthesized Silver Nanoparticles and Leaf Extract of *Caesalpinia bonducella*

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**ABSTRACT**
*Caesalpiniaceae family belongs more than 500 species. A well-known medicinal plant Caesalpinia bonducella belongs to the well-known family, Caesalpiniaceae. Other synonyms are C. crista Linn. and C. bonducella. Flem. The plant is occurred in the subtropical and tropical parts of Asia. It also grows in Nicobar Islands, Andaman, and distributed in all over India. Caesalpinia bonducella (roxb.) shows multifold biological activities which includes anti-inflammatory, antipyretic, antimalarial, anthelmintic, antibacterial, antioxidant, antidiabetic and antitumor activities. Qualitative phytochemical estimation of C. bonducella. indicates the presence of diverse bioactive phytochemicals such as oils, sterols, saponins, alkaloid, glycosides, tannins, phenols, proteins, cardiac glycosides, amino acids, terpenoids, flavonoids, resins and carbohydrates. Antioxidant potential of the water extract & Biosynthesized silver nanoparticles of C. bonducella. leaves were studied using different antioxi4dant methods. The observed antioxidant activity of aqueous extract & Biosynthesized silver nanoparticles was comparable with that of standard BHT in DPPH, ABTS radical scavenging and β-carotene bleaching assays. Biosynthesized silver nanoparticles (CB-AgNPs) showed highest phenolic content (72.00mg GAE/g dry weight) as compared with leaf extract (55.75 mgGAE/g dry weight). The results of studies showed that the leaf extract & biosynthesized silver nanoparticles have considerable antioxidant potential due to bioactive phytochemicals. The present study highlights that biosynthesized silver nanoparticles and leaf extract of C. bonducella. is used as a source of natural antioxidant with its varied medicinal properties. Due to Multifold & correlated activities, Caesalpinia bonducella can be used as antioxidant or reducing agent to synthesize metal nanoparticles. One step synthesis of Silver nanoparticles using water extract of Caesalpinia bonducella is efficient biosynthetic method.*
***Keywords**: C. bonducella., leaves extract, Ag nanoparticles, phytochemical analysis, Antioxidant activity, Total phenolic content.*

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## INTRODUCTION

Caesalpinia (Caesalpiniaceae) is one of the well-known species are distributed in all over India and have infinite source of bioactive compounds. More than 500 species of *C. bonducella.* available worldwide. Other species of Caesalpiniaceae family such as C. sappan, C. Pulcherrima and *C. bonducella.* are distributed thought the world[1]. The plant is distributed in Myanmar, China, Bangladesh, Vietnam and Shrilanka. The plant is commonly distributed in the tropical regions of India. It is also found in the southern, eastern and western parts of India. It mostly occurs in the coastal area of India.[2] Roots, stem, leaves, bark, seeds and nuts of *C. bonducella.* have medicinally useful3. The plant *C. bonducella.* possess Antioxidant[4] , Hepatoprotective[5],[6,[7], Cardio protective8, Analgesic[9,[10], Anti- Inflammatory[9], Antipyretic[11], Antidiabetic[12],[13], Adaptogenic[14], Antimicrobial[15]-[18], Cytotoxicity[19], Anthelmintic[20]-[25], Antifilarial [26], Antiestrogenic[27],[28], Antimalarial[29]-[31], Anticancer [32],[33], Immunomodulatory [34] , Anticonvulsant[35], Antiulcer[36] , Abortifacient[37], Anti-

amyloidogenic**[**38**],** Nootropic[39], Central nervous system activity[40], Larvicidal[41], Hypotensive and antihypertensive[42] , Anti-Angiogenic[43], Hematology and hepatorenal function[44], Anticataract[45], Antispermatogenic[46] properties. *C. bonducella.* seeds shows promising antioxidant activity & contains natural antioxidant agents [33], [34]. The antioxidant potential of *C. bonducella.* was studied by using different antioxidant assays & total phenolic content is also estimated. The chloroform extract shows IC50 value in DPPH assay (170±4.08 µg/ml). Literature survey also supports, extract of *C. bonducella.* has better antioxidant activity [36]. The medicinal plant *C. bonducella.* was found to contain bioactive compounds. These phytochemicals have important role in both reduction of nanoparticles & their stabilization. *C. bonducella.* sees extract have been used for biosynthesis of silver nanoparticles. Cytotoxic & Antibacterial activity of these silver nanoparticles were also studied [47]. The Biosynthesis of nanoparticles using plant extract is beneficial because of simple synthesis and reproducibility. Plant extract-based synthesis of metal nanoparticles involves addition of aqueous extract of plant with appropriate metal salt solution. Aqueous leaf extract of *C. bonducella.* is utilized for silver nanoparticle synthesis. Biosynthesis of *C. bonducella.* Ag nanoparticles take place at room temperature and found to be time dependent. The Biosynthesis of silver nanoparticles is carried out as per the standard methods prescribed in green chemistry. Current chemical and physical methods used for synthesis for metal nanoparticles have disadvantageous like less stability, use of toxic chemicals and handling of these particles. These disadvantageous can be overcome by use of biosynthetic processes using plant extract. Biosynthesized silver nanoparticles using aqueous extract *C. bonducella.* leaf is labelled as CB-AgNPs. This synthesized CB-AgNPs were analyzed by various techniques like UV-visible spectroscopy, FTIR spectroscopy, SEM and EDAX-analysis. The antioxidant evaluation of CB-AgNPs & aqueous extract have been studied using different antioxidant methods like DPPH, ABTS scavenging and β-carotene bleaching assay. *C. bonducella.* plant have medicinal properties, although synthesized nanoparticles (NPs) can be used as antioxidant supplement for health benefits.

## MATERIAL AND METHODS

### Plant material:

Leaves of *C. bonducella.* (2 Kg) were collected from Pathardi, Ahmednagar and authenticated by BSI, Pune. The authentified material is submitted to herbarium store of BSI.

**Abbreviations**: LE; Leaf Extract, AgNPs; Silver nanoparticles, CB; *C. bonducella.*, GAE; Gallic acid equivalent, SEM; Scanning electron microscopy, EDAX; Energy dispersive X-ray analysis, FTIR; Fourier transform infrared spectroscopy.

### Extract Preparation from *C. bonducella.*.

The collected leaves were washed well under running tap water followed by distilled water and then subjected for shade dried. The dried material of leaves was submitted for further analysis. The air-dried leaves of *C. bonducella.* (20g) were cut in to small pieces of the size 0.20 cm X 0.20 cm & taken conical flask. An exact amount of 200 mL of distilled water is added in to the conical flask and heated for about 1 hr. Finally, solution is then filtered by using Whattman filter paper No.44 to get clear and transferent filtrate. The transferent & clear filtrate of water extract of leaves was labeled as LE. The water extract of *C. bonducella.* leaves is stored in freezer at temperature 40C and further utilized for the biosynthesis of silver nanoparticles & antioxidant evaluation.

### Synthesis of Silver nanoparticles from leaves *C. bonducella.*:

Biosynthesis of silver nanoparticles is done by using 4mM AgNO3 solution. To synthesize silver nanoparticles, Leaf extract (LE) of 10.0 mL is added to 100 mL of AgNO3 (4mM) solution. 4 mM AgNO3 is also used as control for the study. Then all the flasks are kept on magnetic stirrer for the process synthesis of AgNPs for about 48 hrs. The flask containing leaf extract & AgNO3 shows formation of silver nanoparticles. These solutions were centrifuged at 10000 rpm for about 30 min. The precipitate was freeze dried and stored in freezer. The synthesized silver nanoparticles was further subjected for characterization & antioxidant studies.

### Characterization of silver nanoparticles

Different characterization techniques like Visual Inspection method, UV-vis, FTIR, FESEM, EDAX were used to characterize biosynthesized nanoparticles. The CB -AgNP was characterized with a double beam UV–VIS spectrophotometer. The scanning range was 200–800 nm with interval of 0.5 nm wavelength. The existence of functional groups in the CB-AgNPs from the leaf extract was done by FTIR spectrometry. Surface morphology of the CB- AgNPs were studied by SEM analysis. EDAX method was used for estimation of elemental composition of CB- AgNPs.

**Antioxidant Activity of Leaf extract (LE) & biosynthesized silver nanoparticles from *C. bonducella.* (CB-AgNPs).**

**Determination of free radical scavenging activity (DPPH)**

The standard method of DPPH assay was used with slight modifications48. Sample & standard BHT solutions of different concentrations (20, 40, 60,100 µg/ml) in methanol were prepared. To each sample & standard solution (1 ml), DPPH solution (0.1mM, 1 ml) was added. Total volume of solution was prepared upto 4 ml using methanol. All these solutions were kept for about 30 minute's incubation in the dark. After 30 minute's incubation absorbance of all solutions were measured at 515 nm. The % inhibition of DPPH radicle by sample/Standard was calculated by using equation:

$$\% \text{ Inhibition} = \frac{[A_C-(A_T- A_B)]}{A_C} \text{ X } 100$$

Where, AC = absorbance of control solution, AT = absorbance of test /standard solutions, AB = absorbance of blank solution.

Antioxidant activity of the samples/Standard is expressed as IC50 values. The Inhibitory concentration (50 %) value is the concentration of sample which inhibits 50 % of DPPH radicals. IC50 values of sample and standard were determined by plotting graph of % Inhibition Vs Concentration.

**Determination of antioxidant activity using β-carotene bleaching assay**

β-carotene bleaching activity was measured using standard protocol with slight modifications49. The concentrations of samples selected for study was 100µg/ml and 500µg/ml. The standard solutions of BHT and BHA (100µg/ml) in methanol were prepared. A control solution was also prepared by adding 0.2 ml methanol and 4 ml of the above emulsion. The blank solution was prepared as described above without β-carotene49. Absorbance of all the samples at 470 nm were taken at zero time and after every 15 mins till the colour of β-carotene disappeared in the control. The % inhibition in β-carotene bleaching activity was calculated by the following equation:

$$\% \text{ Inhibition} = \frac{(A_{A(90)} - A_{C(90)}) \text{ x } 100}{(A_{C(0)} - A_{C(90)})}$$

Where, AA(90) is the absorbance of antioxidants (Sample/Standard) at 90 min., AC(90) is the absorbance of control at 90 min., Ac(0) is the absorbance of control at 0 min.

**ABTS radical cation decolorisation assay**

ABTS assay50 was used to estimate the capacity of leaf extracts and CB-AgNPS of *C. bonducella.* to scavenge the ABTS.+ radical cation. The test solutions of each sample is prepared (100 µg /ml and 500 µg /ml). Standard solutions of BHT and BHA (100 µg /ml) in methanol were also prepared. The control solution was prepared by adding ABTS solution (0.6 ml) to methanol (1.4 ml). The blank was prepared in the same manner as like sample solution but without ABTS solution. Absorbance of all test samples were recorded at 745 nm. All the test samples were examined in triplicates.

$$\% \text{ Inhibition} = \frac{(A_{\text{Control}} - A_{\text{Test}}) \text{ X } 100}{A_{\text{Control}}}$$

**Total Phenolic content**

The total phenolic content of aqueous leaves extract & CB-AGNPs were was examined by using Folin-Ciocalteau method 51. A sample solution of concentration 0.1 mg/ml in methanol was prepared for the estimation of total phenolic content. To 1 ml of each sample solution, 1 ml of Folin-Ciocalteau reagent was added. These solutions were kept at room temperature for about 5 minutes. 10 ml of Na2CO3 (7%) was added to the above sample solutions. These solutions were diluted to 25 ml with distilled water & finally kept for incubation for about 90 min. The blank solution without sample was also prepared in similar way. The absorbance of all sample solutions was measured at 750 nm. Total phenolic content of the samples was expressed as mg gallic acid equivalent / g.

## RESULTS AND DISCUSSION

### Visual Inspection

Initially, a light yellowish color was observed after adding aqueous extract of *C. bonducella.* leaves to solution of AgNO3 (4mM). It is observed that, the colour is changed to dark brown color with time. This confirms synthesis of silver nanoparticles at room temperature. The change in colour is monitored is upto 48 hr.

### UV-vis spectroscopy

UV–visible spectroscopy is primary method used to confirm the formation of silver nanoparticles. The absorption maximum of reaction mixture has been recorded at wavelength 452 nm after 48 hr. This suggests the formation & synthesis of Ag nanoparticles as shown in **Fig.1**. The pattern of the absorption band was symmetrical having spherical-polydispersed nanoparticles.

### FTIR- spectroscopy

The aqueous extract of C. bounducella plant extract acts as reducing agent for synthesized Silver NPs. **Fig.2** shows IR spectra of leaf water extract and synthesized CB-AgNPs. Leaf extract & CB-AgNPs shows band at 3294 cm-1 shows of presence of hydroxyl group of phenolic compounds (Stretching vibrations). Two peaks at 2924 cm-1 and 2854 cm-1 show the stretching of O-H bonds & accumulation of silver ions with the aldehyde group and C-H stretching bonds respectively. IR spectral analysis also shows peak at 1743 cm-1 due to C-N stretching vibrations. EDAX technique is adopted for quantitative estimation of synthesized silver nanoparticles. The amount of Silver was found to be 42 % in EDAX studies. **Fig.3** indicates FESEM images of CB-AgNPs. FESEM studies also indicated aggregation of silver nanoparticles due to adsorption of organic compounds adsorbed on the surface of nanoparticles. These organic moieties may acts capping agent as well as reducing.

### DPPH radical scavenging activity

The DPPH radical scavenging capacity of leaf extracts & CB-AgNPs were expressed in terms of IC50 values. The results are shown in **Fig. 4** & **Fig 5**. BHT and BHA were used as standards for this assay. DPPH radical is stable radicle & acts as hydrogen donors or free radical scavengers. It is commonly used to test the antioxidant nature of plant extracts & compounds. The IC50 value for leaf extract and biosynthesized CB-AgNPs were found to be 72.0 µg /ml (Fig. 4) and 105.2µg /ml (Fig. 5) respectively. Higher IC50 value of test samples in comparison with standard BHT (20 µg/ml) shows that C. bounducella contains antioxidant compounds. This IC50 value of CB-AGNPs shows significant antioxidant activity. It is observed that the DPPH radical antioxidant activity of CB-AgNPS is superior to that of the leaf extract.

### β - Carotene bleaching assay

In this method, oxidation of linoleic acid was inhibited by synthesized CB-AgNPS at both 100µg/ml and 500µg/ml concentration. In β - carotene bleaching assay, % inhibition of test samples & standards were calculated after 90 minutes. Results of the assay are presented in **Fig. 6.** Leaf extract showed moderate activity (59.45 ± 0.27%) and (69.51.2 ± 0.58%) at 100 µg/ml & at 500 µg/ml respectively. An inhibitory activity exhibited by CB-AgNPs are (70.23 ± 0.42%) and (77.97 ± 0.41%) at 100 µg/ml & at 500 µg/ml respectively. The inhibition shown by Leaf extract at 100 µg was less as compared with 500 µg concentration. In this assay the synthesized CB-AgNPs shows superior activity than leaf extracts both concentrations i.e. at 100µg/ml and 500µg/ml.

### ABTS radical cation decolorization assay

The antioxidant capacity of leaf extracts and CB-AgNPs were studied & % inhibition by the test samples & standards in the ABTS method is shown in Fig.7. BHT and BHA were used as standard in this assay. In DPPH & β -carotene methods, CB-AgNPs exhibited very strong antioxidant activity at concentration 500µg /ml. At 500 µg concentration CB-AgNPs exhibited inhibition (80.25±0.15%) which was highest among the test samples and slightly lower than standards BHT & BHA. Leaf extract showed moderate activity (69.51± 0.00%) at 500µg /ml concentration. The inhibition of Leaf extract at 100 µg concentration was found to be 59.45 ± 0.15%.

### Total phenolic contents

CB-AgNPs showed highest phenolic content (72.00mg GAE/g dry weight) as compared with leaf extract (55.75 mg gallic acid equivalent/g dry weight). Inhibition ABTS of & DPPH radical by the leaf extract & CB-AgNPs shows existence of antioxidant compounds in the plant. Among the test samples CB-AgNPs exhibited most potent antioxidant activity than Leaf extract. The difference in antioxidant property of Leaf extract and CB-AgNPs may be due to the variation in relative phenolic content present in them. It was also reported that phenolic compounds are the main cause for antioxidant properties of the extracts51.Present results show that CB-AgNPs has remarkable DPPH and ABTS radical scavenging capacity than leaf extract. CB-AgNPs of *C. bonducella.* can be used as free radicle scavenger.

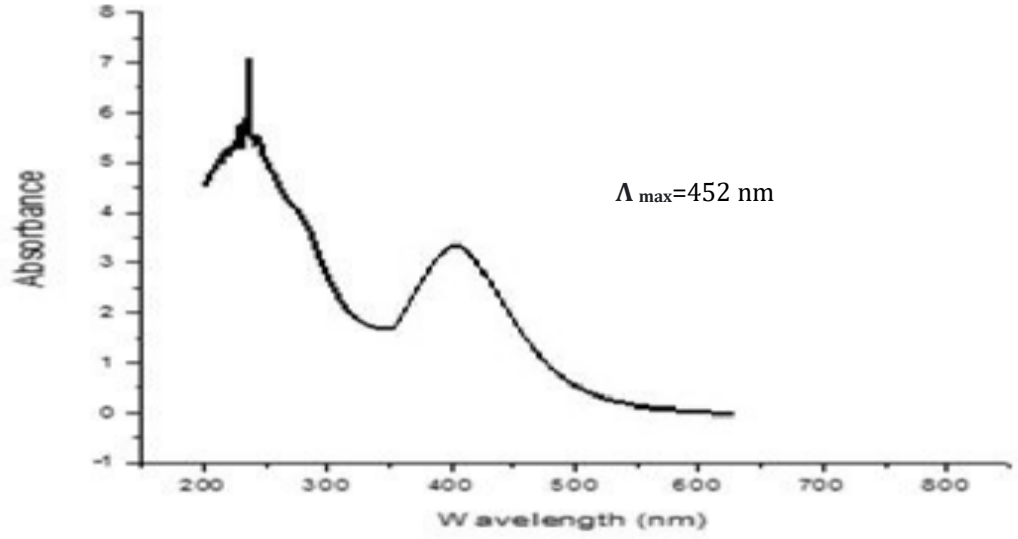


**Fig 1: UV-VIS Spectra of CB-AgNPS at 4mM $AgNO_3$**

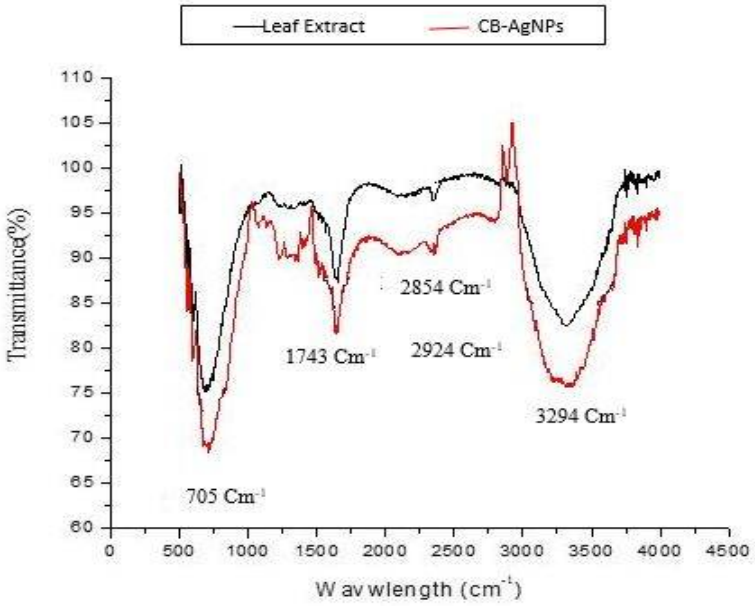


**Fig. 2: IR Spectra of LE and CB-AgNPs**

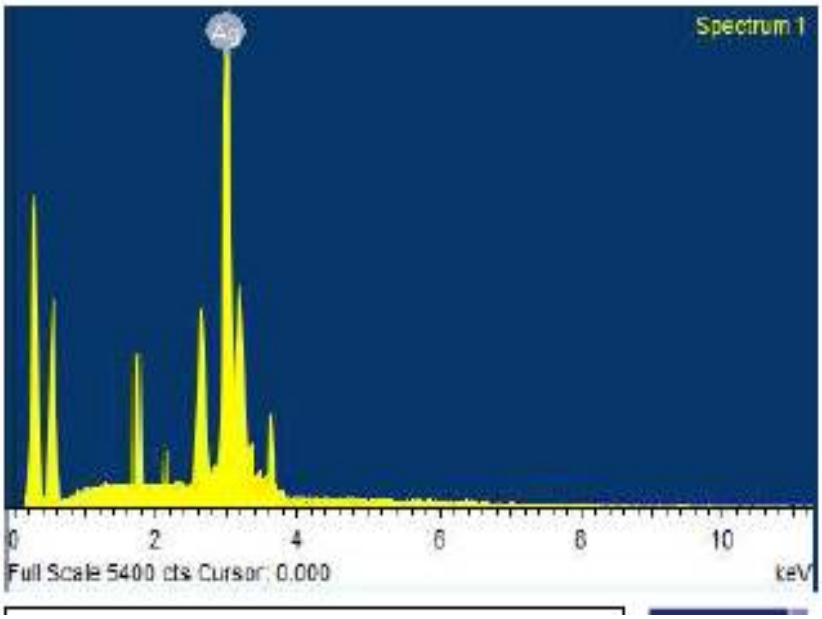


**Fig 3(a): EDAX of CB-AgNPs**

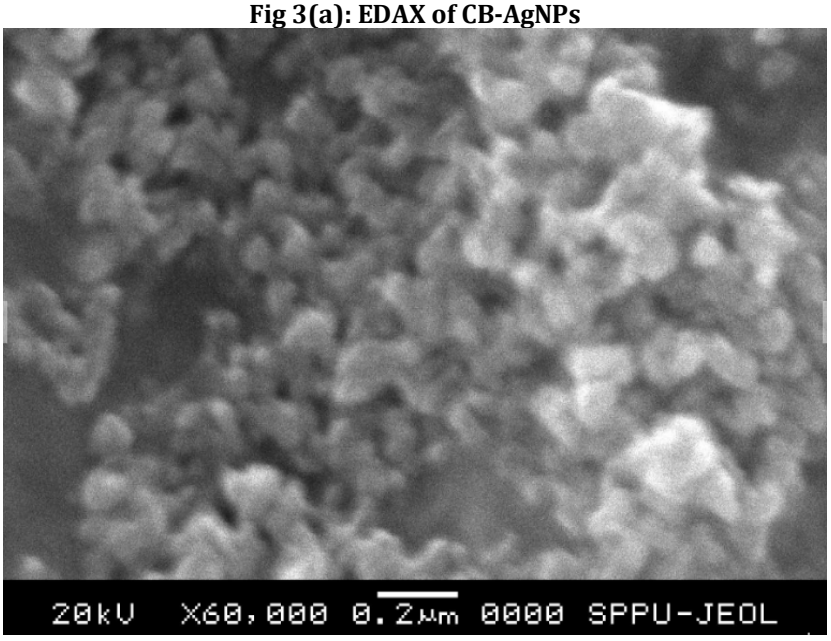


**Fig 3(b): FE SEM of CB-AgNPs**

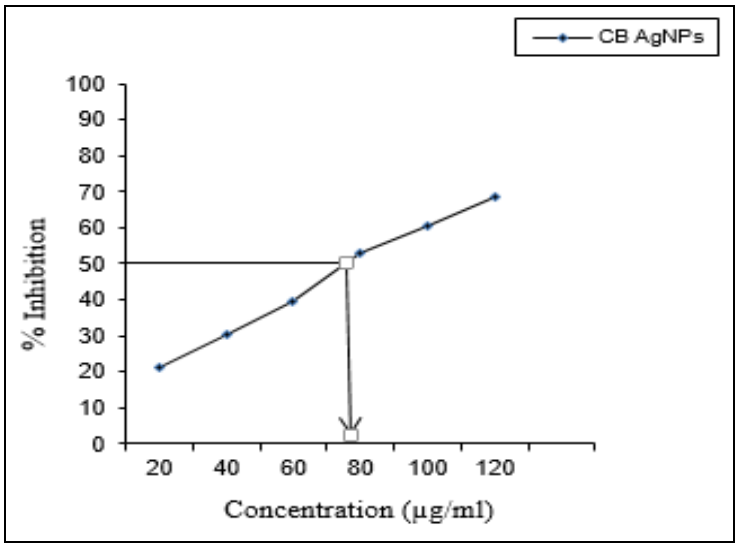


**Fig. 4 : IC50 Graph of Leaf extract of *C. bonducella.***

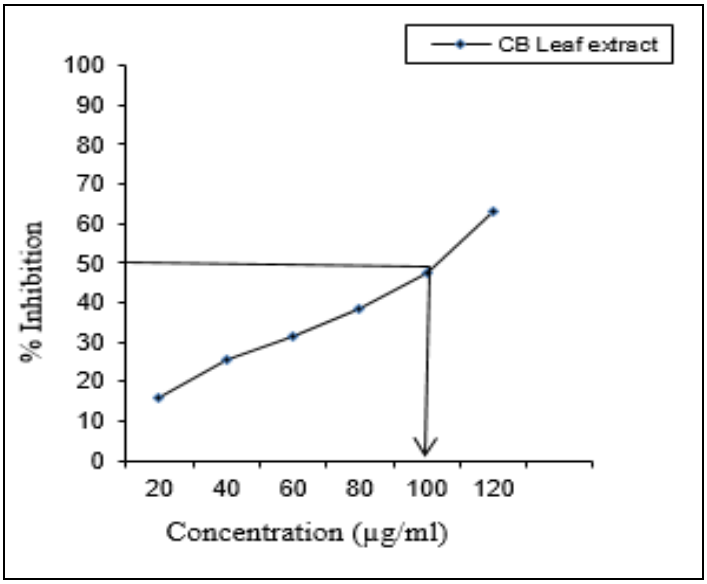


**Fig.5 IC50 Graph of CB –AgNPS synthesized from *C. bonducella.***

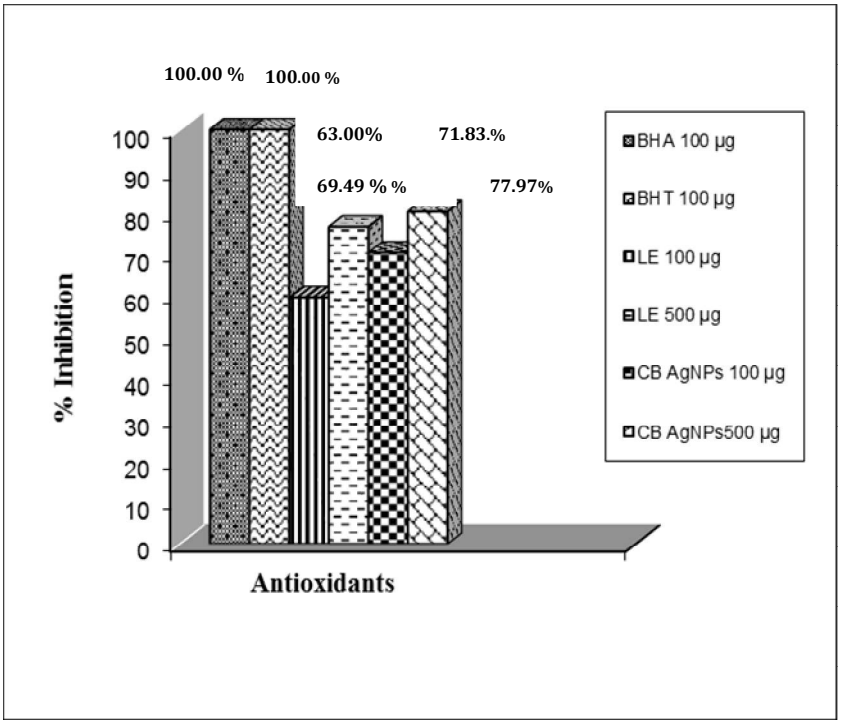


**Fig.6 % Inhibition of Antioxidants in β-carotene bleaching Assay**.

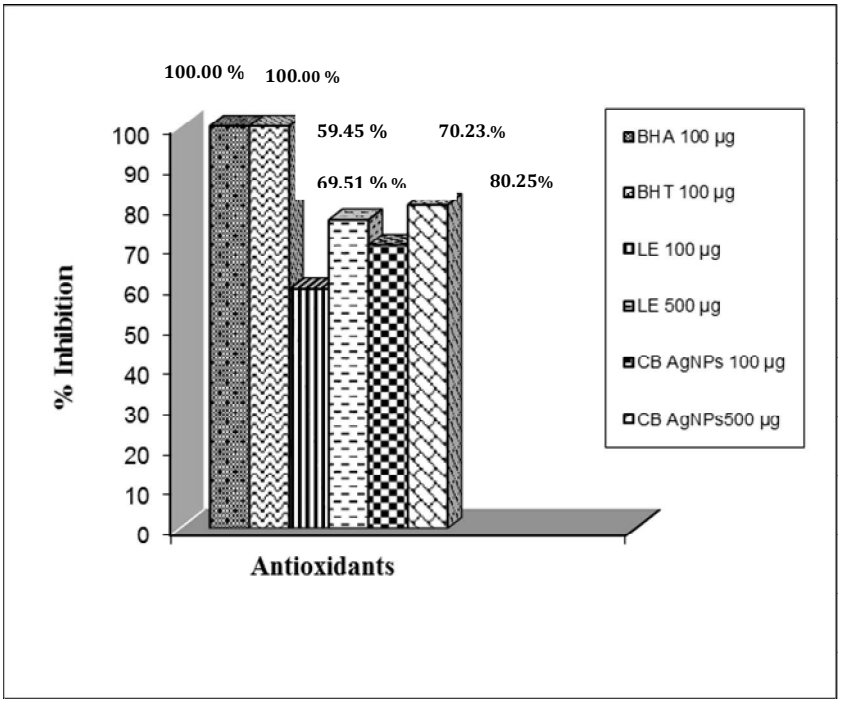


**Fig.7. % Inhibition of Antioxidants in ABTS Assay**

## CONCLUSIONS

Silver nanoparticles were generated by using leaf extract of *C. bonducella.*. The confirmation of the silver nanoparticles was done by UV spectra, FT-IR and SEM analysis. CB-AgNPs nanoparticles shows superior antioxidant activity than leaf extract. These results show that, silver nanoparticles synthesized from *C. bonducella.* leaves can be used as an accessible source of natural antioxidants for health benefits.

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